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Short-chain fatty acids and intestinal inflammation in Multiple Sclerosis – Modulation of female susceptibility by microbial products?

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Abstract

Background. Multiple Sclerosis (MS) is an autoimmune-mediated disease of the central nervous system. Experimental data also suggest a role of intestinal microbiota and microbial products such as short-chain fatty acids (SCFA) in the pathogenesis of MS. A recent clinical study reported beneficial effects (mediated by immunomodulatory mechanisms) after oral administration of the SCFA propionate in MS patients. Based on available evidence, we aimed to investigate whether SCFA and the fecal inflammation marker calprotectin are altered in MS.

Methods. 76 subjects (41 patients with relapsing-remitting MS and 35 age-matched controls) were investigated in this case-control study. All subjects underwent clinical assessment with established scales and provided fecal samples for a quantitative analysis of fecal SCFA and fecal calprotectin concentration. Fecal markers were compared between MS patients and controls, and were analyzed for an association with epidemiological as well as clinical parameters.

Results. Median fecal calprotectin concentrations remained within normal range without any group-specific differences. Fecal SCFA showed a non-significant reduction in MS patients, whereas female subjects showed significantly reduced SCFA concentrations compared to male subjects.

Conclusions. In our cohort of MS patients, we found no evidence of an active intestinal inflammation. As the vast majority of patients, however, was under immunotherapy, this might have affected the outcome measures. The sex-associated difference in fecal SCFA concentrations might at least partially explain female predominance in MS. Large-scale longitudinal studies including drug-naïve MS patients are required to determine the role of SCFA in MS and to distinguish between disease-immanent effects and those caused by the therapeutic regime.

Introduction

Multiple Sclerosis (MS) is an auto-inflammatory disease of the central nervous system (CNS). Autoreactive Th1 and Th17 $CD4^+$ T helper cells and a reduced frequency of regulatory T cells (Tregs) characterize the pro-inflammatory environment in MS (1, 2). The experimental autoimmune encephalomyelitis (EAE) mouse model is the most widely used animal model for MS. Surprisingly, EAE onset is strongly linked to microbial stimuli: colonization of germ-free mice with commensal bacteria leads to EAE development (3), while mice that are kept under germ-free condition do not routinely develop EAE. On the other hand, short-chain fatty acids (SCFA), microbial products mainly produced by intestinal microbiota, counteract demyelination (4). Hence, microbiota, microbial products and the intestinal immune system are likely to be relevant in MS. The SCFA acetate, propionate and butyrate are most abundant in the gut. SCFA are produced by intestinal microbiota via fermentation of dietary fibers. Acetate and propionate derive predominantly from members of the phylum *Bacteroidetes* (such as *Prevotellaceae*) while butyrate mainly originates from bacteria of the phylum *Firmicutes* (such as

Faecalibacterium). Valerate is found in lower concentrations in the feces compared to the more abundant acetate, propionate and butyrate and is considered to derive from different dietary components (5).

Recently, a clinical trial reported a change in the intestinal microbiota accompanied by immunomodulatory effects (inter alia restoration of Treg frequency) and a beneficial clinical effect after oral propionate supplementation in drug-naïve MS patients (6).

In mouse models, SCFA showed pro- and anti-inflammatory effects (7, 8). SCFA can enter the systemic circulation via the intestinal epithelium and cross the blood–brain barrier (8, 2). SCFA interact with immune cells through in different mechanisms such as the NF-kB G-protein coupled receptors-mediated pathway. They lead to epigenetic modulations in T lymphocytes by inhibiting histone deacetylase activity (9), thus leading to higher frequencies of Tregs (10). In turn, Tregs suppress overly active T-cell mediated immune responses. Valerate has been shown to strongly increase IL-10 levels in T- and in regulatory B-cells, a strong immunosuppressive mediator (5). In line with these findings, SCFA are hypothesized to be beneficial in EAE : butyrate has been shown to suppress demyelination and enhance remyelination in mice via oligodendrocyte differentiation (4), while other studies also reported a significant amelioration of EAE by valerate and propionate (5, 11).

Hence, SCFA, which derive from the intestinal microbiota, might be a modulating factor in MS pathology (12).

While a number of experimental data exist, only few studies investigated the intestinal microbiota and SCFA in MS patients. Existing evidence in humans indicates that MS patients suffer from an alteration of their gut microbiota composition (15–17). A Chinese study also reported a decrease in fecal SCFA concentrations (13) and a correlation of fecal SCFA concentrations with Treg frequency in MS patients. Another study reported reduced SCFA blood concentrations in patients with secondary progressive MS (14). Fecal calprotectin (a protein derived from leukocytes that migrate into the intestinal lumen under inflammatory conditions) is a stable and sensitive established marker for inflammatory activity in Crohn’s disease and other inflammatory bowel disease (IBD) (15). Elevated fecal calprotectin concentrations have not only been described in IBD, but also in neurological disorders such as Parkinson’s disease (16, 17).

The limited amount of clinical evidence motivated us to proceed with this case-control study and to investigate fecal calprotectin and fecal SCFA concentrations in relapsing-remitting MS (RRMS) patients.

Subjects And Methods

This case–control study was approved by the local ethics committee (Ethikkommission der Ärztekammer des Saarlandes, registration number 81/18). All subjects provided a signed informed consent form.

76 subjects (41 patients with relapsing-remitting MS and 35 age-matched controls) were assessed between 2018 and 2019 at the Department of Neurology of the Saarland University Medical Center, Germany and the Gesundheitszentrum Glantal, Germany. Inclusion criteria for patients were a diagnosis

of a relapsing-remitting MS (RRMS) according to McDonald's criteria (2017) and being able of signing a written informed consent form. Exclusion criteria for patients and controls were pregnancy, contractual incapacitiy, uncontrolled psychiatric diseases, neurodegenerative disorders, any disease of acute or chronic intestinal inflammation, a coexistent infection within the past four weeks and intake of antibiotics during the past eight weeks. For control subjects, presence or history of any autoimmune-mediated disease was an additional exclusion criterion.

All but three RRMS patients were under immunotherapies (***Table 1***). For analysis of different treatment subgroups, betaferones, glatirameracetate, teriflunomid and dimethylfumarate were defined as *basic therapy*, whereas natalizumab and fingolimod were considered an *escalation therapy*.

All subjects underwent a structured medical history and a clinical examination including scoring with the *Expanded Disability Status Scale* (EDSS), *Constipation Scoring System* (18), *Mini-mental status test*, *Fatigue Impact Scale* and the *Beck Depression Inventory*. All subjects were provided with a fecal sampling kit and instructions on how to collect fecal samples at home as reported previously (19). Fecal SCFA and fecal calprotectin concentrations were quantitatively analyzed as previously described (21, 24).

Data analysis was carried out with IBM SPSS, version 24®. Normality was tested using the Shapiro-Wilk test. Results are reported as median plus range (minimum to maximum). Mann-Whitney-U and Kruskal-Wallis test were used to compare both study groups. Correlation between metric variables was computed using the Pearson's correlation coefficient, while Spearman's correlation coefficient was used to analyze correlations between metric and ordinally scaled parameters. Eta correlation coefficient was finally applied in those cases with metric and nominal variables. Statistical significance was assumed for $p < 0.05$.

Table 1 *summarizes individual epidemiological and clinical data of RRMS patients and controls as well as medians and range (where applicable). Therapeutic regimes defined as* basic therapy *are printed in standard letters, therapeutic regimes defined as* ***escalation therapy*** *are printed in bold italic letters.*

		age (in years)	**sex**	**disease activity**	**therapeutic regime**	**EDSS score**	**CRP** (in mg/l)
RRMS patients	patient 1	28	female	(highly) active	***natalizumab***	2.0	1.3
	patient 2	25	female	mild to moderate	dimethylfumarate	0.0	1.2
	patient 3	36	female	(highly) active	***natalizumab***	2.0	6.0
	patient 4	59	female	(highly) active	***natalizumab***	3.5	1.3
	patient 5	64	female	(highly) active	***natalizumab***	3.5	3.4
	patient 6	62	female	mild to moderate	/	4.0	*
	patient 7	40	female	(highly) active	***natalizumab***	3.0	1.0
	patient 8	47	female	mild to moderate	dimethylfumarate	2.0	3.5
	patient 9	53	female	mild to moderate	glatirameracetate	1.5	*
	patient 10	40	female	(highly) active	***natalizumab***	2.0	1.0
	patient 11	31	female	(highly) active	***natalizumab***	1.0	9.6
	patient	27	female	(highly)	***natalizumab***	3.0	1.0

12			active			
patient 13	50	female	mild to moderate	/	1.5	*
patient 14	68	female	(highly) active	***natalizumab***	3.0	1.7
patient 15	66	female	mild to moderate	β interferon	2.0	1.0
patient 16	45	female	mild to moderate	β interferon	2.5	*
patient 17	31	female	(highly) active	β interferon	3.5	*
patient 18	34	female	mild to moderate	dimethylfumarate	1.5	*
patient 19	68	female	mild to moderate	glatirameracetate	.0	1.0
patient 20	44	female	mild to moderate	glatirameracetate	1.5	*
patient 21	47	female	(highly) active	***natalizumab***	7.0	2.4
patient 22	52	female	(highly) active	***natalizumab***	3.5	1.0
patient 23	56	female	(highly) active	***fingolimod***	3.5	1.5
patient 24	57	female	mild to moderate	glatirameracetate	.0	1.1
patient 25	50	female	(highly) active	***natalizumab***	3.5	1.0

patient 26	58	female	mild to moderate	glatirameracetate	2.0	*
patient 27	37	female	mild to moderate	dimethylfumarate	2.5	3.8
patient 28	56	female	(highly) active	***fingolimod***	1.5	3.5
patient 29	48	female	mild to moderate	glatirameracetate	4.5	*
patient 30	37	male	(highly) active	***natalizumab***	5.0	1.0
patient 31	47	male	mild to moderate	dimethylfumarate	1.5	6.3
patient 32	54	male	(highly) active	dimethylfumarate	2.5	*
patient 33	60	male	mild to moderate	glatirameracetate	3.0	*
patient 34	47	male	(highly) active	***fingolimod***	5.0	1.6
patient 35	50	male	(highly) active	***fingolimod***	2.0	1.0
patient 36	41	male	mild to moderate	/	.0	*
patient 37	22	male	(highly) active	***natalizumab***	1.0	1.0
patient 38	48	male	(highly) active	β interferon	3.0	1.5

	patient 39	59	male	mild to moderate	β interferon	2.0	*
	patient 40	54	male	(highly) active	***fingolimod***	3.0	*
	patient 41	37	male	(highly) active	***fingolimod***	3.5	2.0
	median range	48 22 to 68	n/a	n/a	n/a	2.5 0 to 7	19 19 to 141
control subjects	control 1	72	female				5.8
	control 2	31	female				1.0
	control 3	60	female				1.6
	control 4	59	female				3.7
	control 5	47	female				1.0
	control 6	56	female				6.9
	control 7	48	female				11.0
	control 8	23	female				*
	control	29	female				1.4

9						
control 10	60	female				1.0
control 11	56	female				1.0
control 12	58	female				1.8
control 13	36	female				1.0
control 14	50	male				1.4
control 15	43	male				1.0
control 16	47	male				2.6
control 17	58	male				*
control 18	58	male				1.0
control 19	57	male				6.0
control 20	56	male				1.0
control 21	61	male				2.7
control 22	67	male				1.0

control 23	30	male				14.0
control 24	27	male				*
control 25	53	male				1.0
control 26	30	male				11.0
control 27	40	male				*
control 28	39	male				*
control 29	27	male				2.2
control 30	48	male				1.0
control 31	61	male				1.1
control 32	29	male				1.0
control 33	29	male				1.0
control 34	56	male				1.1
control 35	28	male				*

	median **range**	48 23 to 72	n/a	n/a	n/a	n/a	19 19 to 328

Results

Demographic and clinical data

RRMS patients (n=41) and controls (n=35) were matched for age (median age RRMS patients: 48 years, range 22 to 68 years; median age controls: 48 years, range 23 to 72 years). There were more female subjects in the RRMS group (29 of 41 subjects) EDSS scores were significantly higher in RRMS patients with an active / highly active RRMS (n=23, median: 3, range 1 to 7) compared to those with a mild / moderate disease activity (n=18, median: 2.5, range 0 to 7, p 0.004). Blood CRP concentrations as a marker of systemic inflammation were not part of the study protocol, but available for most subjects (29 of 35 controls, 27 of 41 RRMS patients). None of the subjects enrolled in this case-control study showed a clinically relevant increase in CRP concentration. All epidemiological and clinical data are summarized in ***Table 1***.

Fecal calprotectin concentrations

No significant difference existed between the fecal calprotectin concentration of RRMS patients (median: 19 µg/g, range 19 –141 µg/g) and healthy controls (median: 19 µg/g, range 19–328 µg/g) as shown in ***Figure 1***. Concentration remained within normal range in both groups. There was no difference in fecal calprotectin concentrations between RRMS patients under basic therapy compared to escalation therapy, between mild disease compared to (highly) active disease and no difference between different drugs (data not shown). No significant difference in fecal calprotectin concentrations between both sex could be observed.

Figure 1 *Fecal calprotectin in patients and controls visualized as boxplot. The control group contains an outlier with a fecal calprotectin concentration of 328 µg/g, not depicted for better visualization.*

Fecal short-chain fatty acid concentrations

There was no difference in fecal SCFA concentrations between RRMS patients and controls (***Supplemental Table 1, Supplemental Figure 1***). Median fecal butyrate concentration, however, was reduced by 77 % in RRMS patients (p 0.219). Median fecal acetate concentration was descriptively reduced by 72 % in patients with an active / highly active RRMS compared to those with a mild / moderate activity (p 0.554) (***Supplemental Figure 2***).

Aside from the two branched-chain SCFA iso-valerate and iso-butyrate, all SCFA concentrations were significantly lower in women compared to men (***Table 2a, Figure 2***). Analyzing RRMS patients and controls separately, acetate, propionate and butyrate were significantly lower in women compared to men in the control group (***Table 2b***); for RRMS patients, there was no statistical significance but a similar trend (***Table 2c***).

Table 2 *shows fecal SCFA concentrations (in mmol/g, median and range) and the respective p value (difference between female and male subjects) for each investigated SCFA separately.* ***Table 2a*** *shows all subjects.* ***Table 2b*** *shows controls.* ***Table 2c*** *shows RRMS patients.*

		acetate mmol/g	propionate mmol/g	butyrate mmol/g	iso-butyrate mmol/g	valerate mmol/g	iso-valerate mmol/g
female subjects (n=42)	median	6.47	1.44	1.37	0.88	0.53	0.55
	minimum	0.07	0.10	0.02	0.00	0.00	0.01
	maximum	160.26	43.07	41.49	6.13	5.64	5.49
male subjects (n=34)	median	68.89	19.40	15.63	2.10	1.58	1.94
	minimum	0.67	0.22	0.05	0.02	0.01	0.02
	maximum	193.06	99.47	52.54	11.35	19.76	17.03
p		**0.012**	**0.002**	**0.003**	0.061	**0.004**	0.068

		acetate in mmol/g	propionate in mmol/g	butyrate in mmol/g	iso-butyrate in mmol/g	valerate in mmol/g	iso-valerate in mmol/g
female controls (n=13)	median	8.02	1.59	1.89	1.55	.63	2.04
	minimum	.66	.17	.08	.01	.01	.01
	maximum	71.22	27.08	18.98	4.01	3.05	5.32
male controls (n=22)	median	73.4	21.09	15.95	2.33	2.07	2.25
	minimum	1.04	.22	.05	.02	.01	.02
	maximum	193.06	87.81	.52.54	11.35	19.76	17.03
p		**0.005**	**0.004**	0.16	0.243	0.067	0.335

		acetate in mmol/g	propionate in mmol/g	butyrate in mmol/g	iso-butyrate in mmol/g	valerate in mmol/g	iso-valerate in mmol/g
female patients (n=29)	median	3.76	1.29	.88	.97	.96	.099
	minimum	.07	.1	.02	.004	.002	.007
	maximum	160.26	43.07	41.49	6.13	5.64	5.49
male patients (n=12)	median	51.48	14.25	9.17	1.9	1.55	1.6
	minimum	.67	.37	.14	.02	.01	.02
	maximum	121.05	99.47	39.53	4.23	5.2	5.87
p		0.357	0.227	0.127	0.436	0.142	0.342

All fecal SCFA concentrations showed a statistically significant negative correlation with age (***Table 3*** and ***Supplemental Figure 3)***.

Table 3 *shows the Pearson's correlation coefficient and respective p values for the correlation between age and fecal SCFA concentrations.* ***Table 3b*** *shows controls.* ***Table 3c*** *shows RRMS patients.*

subjects (n=76)	acetate	propionate	butyrate	iso-butyrate	valerate	iso-valerate
Pearson's correlation coefficient	-0.335	-0.268	-0.335	-0.279	-0.316	-0.275
p	**0.003**	**0.019**	**0.003**	**0.015**	**0.005**	**0.016**

controls (n=35)	acetate	propionate	butyrate	iso-butyrate	valerate	iso-valerate
Pearson’s correlation coefficient	-0.522	-0.483	-0.480	-0.452	-0.444	-0.434
p	**0.001**	**0.003**	**0.003**	**0.006**	**0.006**	**0.009**

RRMS patients (n=41)	acetate	propionate	butyrate	iso-butyrate	valerate	iso-valerate
Pearson’s correlation coefficient	-0.128	-0.026	-0.297	-0.043	-0.088	-0.01
p	0.435	0.874	0.297	0.791	0.584	0.952

Discussion

Experimental studies suggest that microbiota, microbial products (like SCFA) and the intestinal immune system might be involved in the pathogenesis of MS. Hitherto, only sparse clinical data exist. In this study, we investigated fecal markers related to intestinal inflammation in RRMS patients and age-matched controls.

Contrary to what we initially hypothesized, fecal calprotectin concentrations, a robust and sensitive marker even for subclinical intestinal inflammation, was in the normal range in the majority of investigated RRMS patients and there was no difference regarding fecal calprotectin concentrations between RRMS and control subjects. While there is one study reporting elevated calprotectin concentrations in the cerebrospinal fluid of MS patients (20), fecal calprotectin concentrations have not been reported for MS previously. Normal fecal calprotectin concentrations could be caused by the fact that most investigated RRMS patients were under immunotherapy, which beside their effect on the CNS alter enteric inflammatory processes, as well. Consequently, the observation of normal fecal calprotectin concentrations in our RRMS cohort might be explained by this effect, in particular 14 of the RRMS patients were treated with natalizumab, a drug also administered in Crohn’s disease(21).

Assuming that immunotherapies in MS exert anti-inflammatory effects also in the gastrointestinal tract, the intestinal microbiota (as indicated by Storm-Larsen et al. for dimethylfumarate (27)) and

subsequently intestinal SCFA production might be affected as well. Hence, the lack of a significant difference between RRMS patients and controls with regard to fecal SCFA concentrations in this study might also be explained by a drug effect.

Despite the lack of a statistical significance, we observed descriptively lower fecal SCFA concentrations in RRMS patients compared to controls, especially for butyrate. This descriptive finding is in line with the few studies investigating SCFA in MS: Park et al. showed, that SCFA blood concentrations were reduced in MS patients (14). A Chinese study reported reduced fecal SCFA concentrations in MS patients (13). An altered intestinal microbiota has been reported in MS patients as well (22–24). Moreover, the highly significant correlation of fecal SCFA concentrations with age in controls, but not in patients, endorses the assumption that either MS or MS therapeutics affect gut microbiota metabolism.

Recently, the potential clinical relevance of SCFA in MS has been investigated in a clinical trial (6): Duscha et al. reported an enhancement of Treg differentiation, reduced autoinflammation and improvements in the clinical course of MS after oral administration of propionate (6). It is important to note that orally administered SCFA are absorbed to a great extent in the small intestine. SCFA produced by the gut microbiota in the colon mainly exert local effects and are unlikely to affect systemic SCFA concentrations as effective as an oral supplementation.

We are not able to draw conclusions concerning fecal calprotectin and SCFA concentrations in drug-naïve MS patients as the vast majority of our RRMS cohort was under immunotherapy. As the investigated RRMS patients were under different treatment regimes, we also analyzed subgroups of RRMS patients defined by the therapeutic regime. Yet, the number of subjects per subgroup was rather small and the study population was not treated with the full spectrum of available MS therapies. Large-scale longitudinal studies, including drug-naïve MS patients are necessary to distinguish between disease-immanent and therapeutic effects on intestinal inflammation, intestinal microbiota and microbial products, like SCFA, in MS. Another interesting topic for future investigations is the role of (subclinical) intestinal inflammation as a trigger for relapse in MS

An unexpected finding of our study was the marked sex-associated difference in SCFA concentration between women and men with significantly lower SCFA concentrations in female subjects. Sex-specific differences have been described for the intestinal microbiota previously (25). Fecal SCFA concentrations have already been subject of clinical studies in different fields, e.g. anorexia (29), obesity, diabetes mellitus and cardiometabolic disease (30). Yet, none of these studies reported sex-specific differences for fecal SCFA concentrations. It might well be that this aspect was not explicitly analyzed in these studies.

Jakobsdottir and colleagues reported sex-specific differences of blood SCFA concentrations (with lower SCFA concentrations in female subjects) in a study comparing patients with microscopic colitis and celiac disease (26). Another study did not find sex-specific differences when analyzing blood SCFA concentrations (27). As already mentioned, blood and fecal SCFA concentrations are not directly comparable.

In addition, potential confounding factors such as dietary habits, need also to be investigated. Patients and controls in this study were matched in terms of age, but there was a male predominance in the control group, which represents a potential confounder.

Taken together, the known female predominance in MS and the known immunomodulatory effects of SCFA warrant further studies in this field. One might hypothesize that low concentrations of SCFA represent an additional risk factor for MS and might contribute to the higher susceptibility of women compared to men in MS. As the observed sex-specific difference in SCFA concentrations was independent from MS, also studies in other conditions that investigate microbiota and microbial products should consider sex as a potential confounder.

List Of Abbreviations

CNS – central nervous system

EAE – experimental autoimmune encephalomyelitis

MS – Multiple sclerosis

RRMS – relapsing remitting multiple sclerosis

SCFA – short-chain fatty acids

SD – standard deviation

ST – student's t-test (unpaired)

Tregs – regulatory T cells

Declarations

Competing interests

The authors state that here is no conflict of interest, financial or otherwise, related to the submitted work.

Consent for publication

Not applicable.

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Data availability

The datasets supporting the conclusions of this article are included within the article and its additional files.

Ethic approval

The study was approved by the local ethics committee committee (Ethikkommission der Ärztekammer des Saarlandes, registration number 81/18). Written informed consent was obtained from all participants.

Authors' contributions

AB conceived essential aspects of the data analyses, performed these analyses and drafted the manuscript. MA assisted in creating the study protocol, enrolled and examined all subjects (study-related procedures), created a database for analysis, contributed to statistical analysis and revised the manuscript. MF and MU created the study protocol, supervised the study and revised the manuscript. AS performed laboratory analysis and revised the manuscript. KF and SW provided critical feedback to the study design and the manuscript.

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Figures

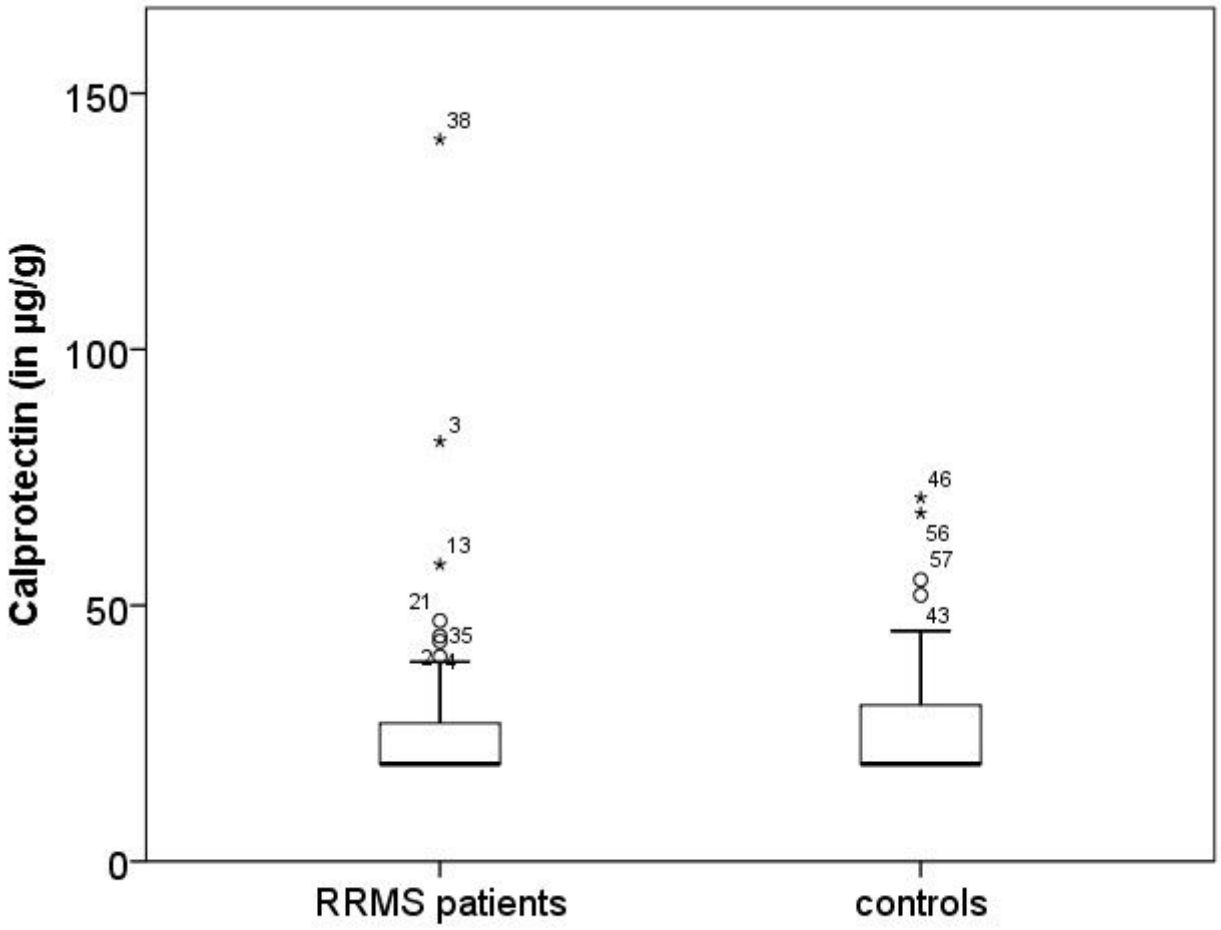


Figure 1

Fecal calprotectin in patients and controls visualized as boxplot. The control group contains an outlier with a fecal calprotectin concentration of 328 µg/g, not depicted for better visualization.

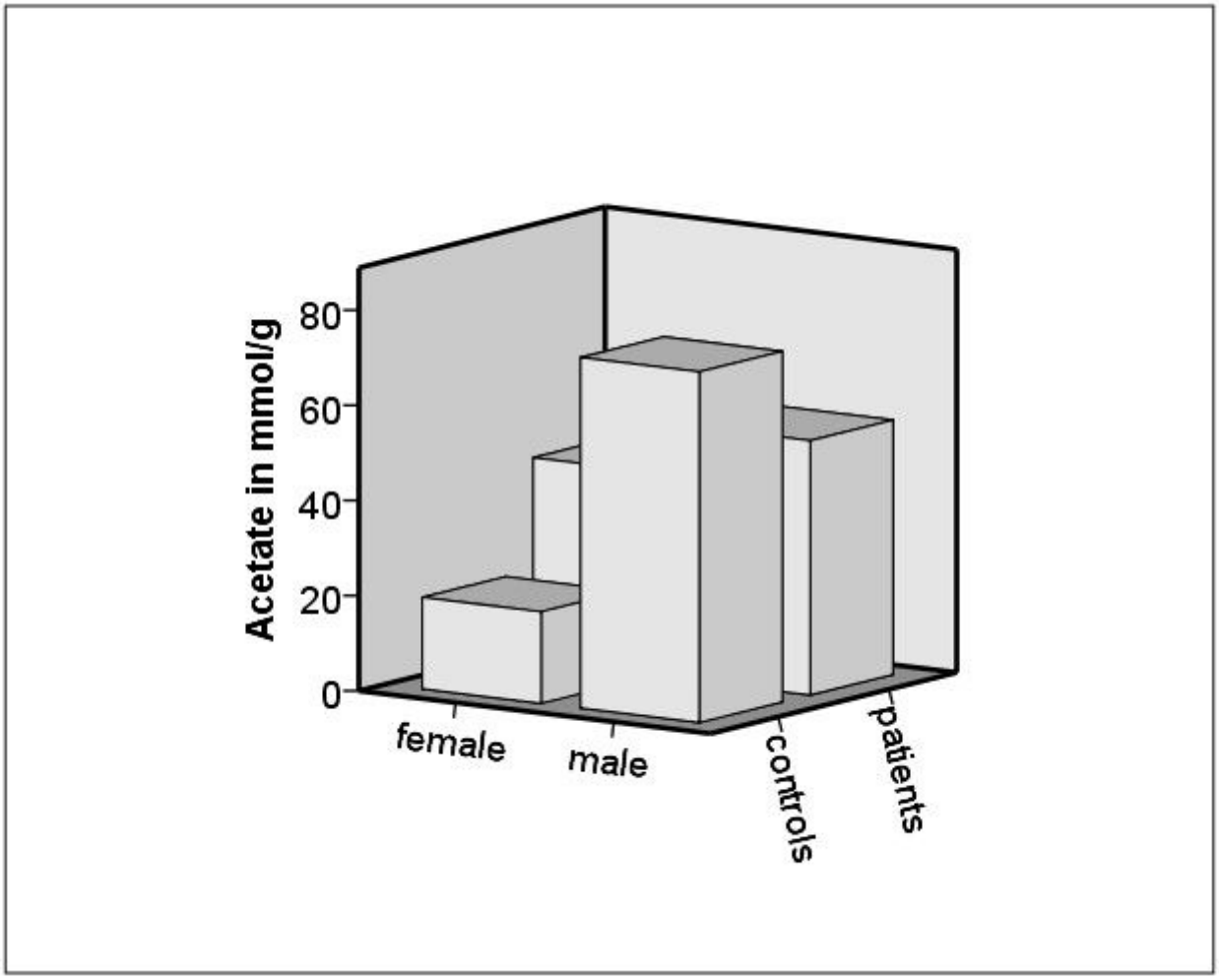


Figure 2

Fecal acetate in female and male patients and controls visualized as bar chart.

Supplementary Files

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- SuppFig1abc.png
- SuppFig2.jpg
- SuppFig3.jpg
- SupplementaryTable1.docx